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(71) Applicant: THERASENSE, INC. [US/US]; 1360 South Loop Road, Alameda, CA 94502 (US).

(72) Inventors: MAO, Fei; 34379 Benedick Lane, Fremont, CA 94555 (US). HELLER, Adam; 4711 Spicewood Springs Road, Apt. 271, Austin, TX 78759 (US).

(74) Agent: DAIGNAULT, Ronald, A.; Merchant & Gould P.C., P.O. Box 2903, Minneapolis, MN 55402-0903 (US).

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(54) Title: TRANSITION METAL COMPLEXES WITH BIDENTATE LIGAND HAVING AN IMIDAZOLE RING

(57) Abstract: Novel transition metal complexes of iron, cobalt, ruthenium, osmium, and vanadium are described. The transition metal complexes can be used as redox mediators in enzyme based electrochemical sensors. In such instances, transition metal complexes accept electrons from, or transfer electrons to, enzymes at a high rate and also exchange electrons rapidly with the sensor. The transition metal complexes include at least one substituted or unsubstituted biimidazole ligand and may further include a second substituted or unsubstituted biimidazole ligand or a substituted or unsubstituted bipyridine or pyridylimidazole ligand. Transition metal complexes attached to polymeric backbones are also described.

TRANSITION METAL COMPLEXES WITH BIDENTATE LIGAND
HAVING AN IMIDAZOLE RING

This application is being filed as a PCT International Patent application in the name of TheraSense, Inc., a U.S. national corporation, on 14 November 2000, designating all countries except the United States of America.

Field of the Invention

This invention relates to transition metal complexes with at least one bidentate ligand containing at least one imidazole ring. In addition, the invention relates to the preparation of the transition metal complexes and to the use of the transition metal complexes as redox mediators.

Background of the Invention

Enzyme based electrochemical sensors are widely used in the detection of analytes in clinical, environmental, agricultural and biotechnological applications. Analytes that can be measured in clinical assays of fluids of the human body include, for example, glucose, lactate, cholesterol, bilirubin and amino acids. Levels of these analytes in biological fluids, such as blood, are important for the diagnosis and the monitoring of diseases.

Electrochemical assays are typically performed in cells with two or three electrodes, including at least one measuring or working electrode and one reference electrode. In three electrode systems, the third electrode is a counter-electrode. In two electrode systems, the reference electrode also serves as the counter-electrode. The electrodes are connected through a circuit, such as a potentiostat. The measuring or working electrode is a non-corroding carbon or metal conductor. Upon passage of a current through the working electrode, a redox enzyme is electrooxidized or electroreduced. The enzyme is specific to the analyte to be detected, or to a product of the analyte. The turnover rate of the enzyme is typically related (preferably, but not necessarily, linearly) to the concentration of the analyte itself, or to its product, in the test solution.

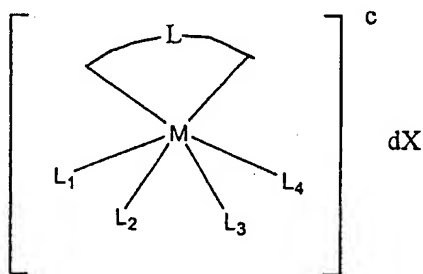
The electrooxidation or electroreduction of the enzyme is often facilitated by the presence of a redox mediator in the solution or on the electrode. The redox mediator assists in the electrical communication between the working electrode and the enzyme. The redox mediator can be dissolved in the fluid to be analyzed, which is in electrolytic contact with the electrodes, or can be applied within a coating on the working electrode in electrolytic contact with the analyzed solution. The coating is preferably not soluble in water, though it may swell in water. Useful devices can be made, for example, by coating an electrode with a film that includes a redox mediator and an enzyme where the enzyme is catalytically specific to the desired analyte, or its product. In contrast to a coated redox mediator, a diffusional redox mediator, which can be soluble or insoluble in water, functions by shuttling electrons between, for example, the enzyme and the electrode. In any case, when the substrate of the enzyme is electrooxidized, the redox mediator transports electrons from the substrate-reduced enzyme to the electrode; when the substrate is electroreduced, the redox mediator transports electrons from the electrode to the substrate-oxidized enzyme.

Recent enzyme based electrochemical sensors have employed a number of different redox mediators such as monomeric ferrocenes, quinoid-compounds including quinines (e.g., benzoquinones), nickel cyclamates, and ruthenium amines. For the most part, these redox mediators have one or more of the following limitations: the solubility of the redox mediators in the test solutions is low, their chemical, light, thermal, or pH stability is poor, or they do not exchange electrons rapidly enough with the enzyme or the electrode or both. Additionally, the redox potentials of many of these reported redox mediators are so oxidizing that at the potential where the reduced mediator is electrooxidized on the electrode, solution components other than the analyte are also electrooxidized; in other cases they are so reducing that solution components, such as, for example, dissolved oxygen are also rapidly electroreduced. As a result, the sensor utilizing the mediator is not sufficiently specific.

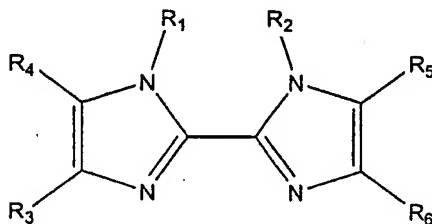
Summary of the Invention

The present invention is directed to novel transition metal complexes. The present invention is also directed to the use of the complexes as redox mediators. The preferred redox mediators typically exchange electrons rapidly with enzymes and electrodes, are stable, and have a redox potential that is tailored for the electrooxidation of analytes, exemplified by glucose.

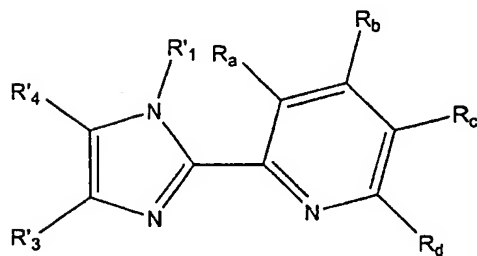
One embodiment of the invention is a transition metal complex having the formula:



- 10 M is cobalt, ruthenium, osmium, or vanadium. L is selected from the group consisting of:

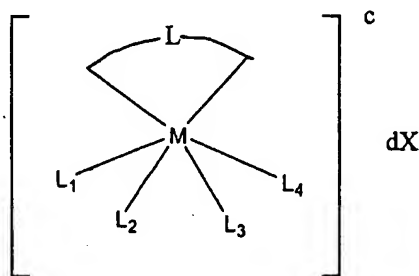


and



R_1 , R_2 , and R'_1 are independently substituted or unsubstituted alkyl, alkenyl, or aryl groups. R_3 , R_4 , R_5 , R_6 , R'_3 , R'_4 , R_a , R_b , R_c , and R_d are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, aryl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl. c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge. X represents at least one counter ion and d is an integer from 1 to 5 representing the number of counter ions, X . L_1 , L_2 , L_3 and L_4 are other ligands.

Another embodiment is a redox mediator having the formula:

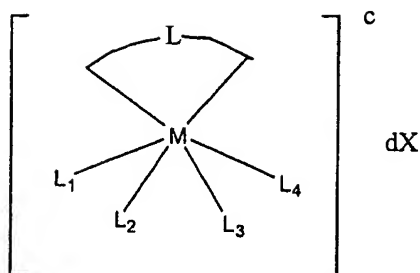


M is iron, cobalt, ruthenium, osmium, or vanadium. L is a bidentate ligand comprising at least one imidazole ring. c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge. X represents at least one counter ion and d is an integer from 1 to 5 representing the number of counter ions, X . L_1 , L_2 , L_3 and L_4 are other ligands.

Another embodiment is a sensor that includes the redox polymer, a working electrode, and a counter electrode. The redox polymer is disposed proximate to the working electrode.

Yet another embodiment is a polymer that includes a polymeric backbone and a transition metal complex having the following formula:

5



- M is iron, cobalt, ruthenium, osmium, or vanadium. L is a bidentate ligand comprising at least one imidazole ring. c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge. X represents at least one counter ion and d is an integer from 1 to 5 representing the number of counter ions, X. L₁, L₂, L₃ and L₄ are other ligands where at least one of L, L₁, L₂, L₃ and L₄ couples to the polymeric backbone.

Detailed Description

When used herein, the following definitions define the stated term:

- The term "alkyl" includes linear or branched, saturated aliphatic hydrocarbons. Examples of alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, *tert*-butyl and the like. Unless otherwise noted, the term "alkyl" includes both alkyl and cycloalkyl groups.

- The term "alkoxy" describes an alkyl group joined to the remainder of the structure by an oxygen atom. Examples of alkoxy groups include methoxy, ethoxy, n-propoxy, isopropoxy, butoxy, *tert*-butoxy, and the like. In addition, unless otherwise noted, the term 'alkoxy' includes both alkoxy and cycloalkoxy groups.

- The term "alkenyl" describes an unsaturated, linear or branched aliphatic hydrocarbon having at least one carbon-carbon double bond. Examples of alkenyl groups include ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-methyl-1-propenyl, and the like.

- A "reactive group" is a functional group of a molecule that is capable of reacting with another compound to couple at least a portion of that other compound to the molecule. Reactive groups include carboxy, activated ester, sulfonyl halide, sulfonate ester, isocyanate, isothiocyanate, epoxide, aziridine, halide, aldehyde, ketone, amine, acrylamide, thiol, acyl azide, acyl halide, hydrazine, hydroxylamine,

alkyl halide, imidazole, pyridine, phenol, alkyl sulfonate, halotriazine, imido ester, maleimide, hydrazide, hydroxy, and photo-reactive azido aryl groups. Activated esters, as understood in the art, generally include esters of succinimidyl, benzotriazolyl, or aryl substituted by electron-withdrawing groups such as sulfo, nitro, cyano, or halo groups; or carboxylic acids activated by carbodiimides.

A "substituted" functional group (e.g., substituted alkyl, alkenyl, or alkoxy group) includes at least one substituent selected from the following: halogen, alkoxy, mercapto, aryl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, -NH₂, alkylamino, dialkylamino, trialkylammonium, alkanoylamino, arylcarboxamido, hydrazino, alkylthio, alkenyl, and reactive groups.

A "biological fluid" is any body fluid or body fluid derivative in which the analyte can be measured, for example, blood, interstitial fluid, plasma, dermal fluid, sweat, and tears.

An "electrochemical sensor" is a device configured to detect the presence of or measure the concentration or amount of an analyte in a sample via electrochemical oxidation or reduction reactions. These reactions typically can be transduced to an electrical signal that can be correlated to an amount or concentration of analyte.

A "redox mediator" is an electron transfer agent for carrying electrons between an analyte or an analyte-reduced or analyte-oxidized enzyme and an electrode, either directly, or via one or more additional electron transfer agents.

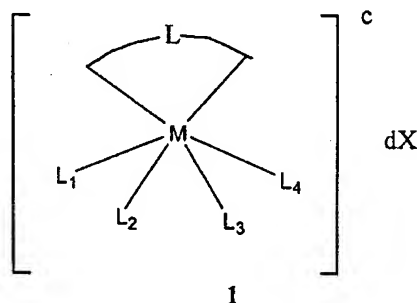
"Electrolysis" is the electrooxidation or electroreduction of a compound either directly at an electrode or via one or more electron transfer agents (e.g., redox mediators or enzymes).

The term "reference electrode" includes both a) reference electrodes and b) reference electrodes that also function as counter electrodes (i.e., counter/reference electrodes), unless otherwise indicated.

The term "counter electrode" includes both a) counter electrodes and b) counter electrodes that also function as reference electrodes (i.e., counter/reference electrodes), unless otherwise indicated.

Generally, the present invention relates to transition metal complexes of iron, cobalt, ruthenium, osmium, and vanadium having at least one bidentate ligand

containing an imidazole ring. The invention also relates to the preparation of the transition metal complexes and to the use of the transition metal complexes as redox mediators. In at least some instances, the transition metal complexes have one or more of the following characteristics: redox potentials in a particular range, the ability to exchange electrons rapidly with electrodes, the ability to rapidly transfer electrons to or rapidly accept electrons from an enzyme to accelerate the kinetics of electrooxidation or electroreduction of an analyte in the presence of an enzyme or another analyte-specific redox catalyst. For example, a redox mediator may accelerate the electrooxidation of glucose in the presence of glucose oxidase or PQQ-glucose dehydrogenase, a process that can be useful for the selective assay of glucose in the presence of other electrochemically oxidizable species. Compounds having the formula 1 are examples of transition metal complexes of the present invention.

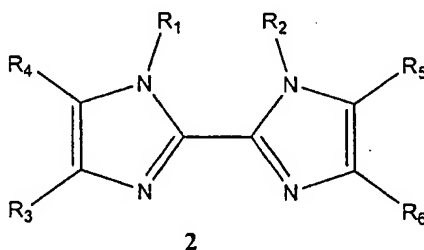


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M is a transition metal and is typically iron, cobalt, ruthenium, osmium, or vanadium. Ruthenium and osmium are particularly suitable for redox mediators.

L is a bidentate ligand containing at least one imidazole ring. One example of L is a 2,2'-biimidazole having the following structure 2:

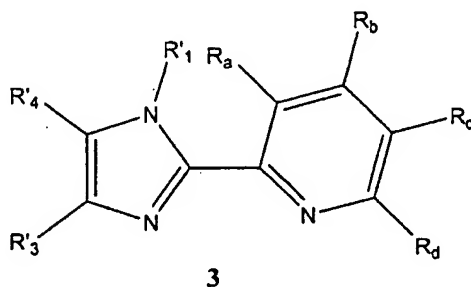
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R_1 and R_2 are substituents attached to two of the 2,2'-biimidazole nitrogens and are independently substituted or unsubstituted alkyl, alkenyl, or aryl groups. Generally, R_1 and R_2 are unsubstituted C1 to C12 alkyls. Typically, R_1 and R_2 are unsubstituted C1 to C4 alkyls. In some embodiments, both R_1 and R_2 are methyl.

- 5 R_3 , R_4 , R_5 , and R_6 are substituents attached to carbon atoms of the 2,2'-biimidazole and are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl.
- 10 Alternatively, R_3 and R_4 in combination or R_5 and R_6 in combination independently form a saturated or unsaturated 5- or 6-membered ring. An example of this is a 2,2'-bibenzoimidazole derivative. Typically, the alkyl and alkoxy portions are C1 to C12. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl
- 15 portions), alkoxy, alkylthio, aryl, or a reactive group. Generally, R_3 , R_4 , R_5 , and R_6 are independently -H or unsubstituted alkyl groups. Typically, R_3 , R_4 , R_5 , and R_6 are -H or unsubstituted C1 to C12 alkyls. In some embodiments, R_3 , R_4 , R_5 , and R_6 are all -H.

- Another example of L is a 2-(2-pyridyl)imidazole having the following
- 20 structure 3:



R'_1 is a substituted or unsubstituted aryl, alkenyl, or alkyl. Generally, R'_1 is a substituted or unsubstituted C1-C12 alkyl. R'_1 is typically methyl or a C1-C12 alkyl that is optionally substituted with a reactive group.

- 25 R'_3 , R'_4 , R'_a , R'_b , R'_c , and R'_d are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino,

alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl. Alternatively, R_c and R_d in combination or R'_3 and R'_4 in combination can form a saturated or unsaturated 5- or 6-membered ring. Typically, the alkyl and alkoxy portions are C1 to C12. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. Generally, R'_3 , R'_4 , R_a , R_b , R_c and R_d are independently -H or unsubstituted alkyl groups. Typically, R_a and R_c are -H and R'_3 , R'_4 , R_b , and R_d are -H or methyl.

c is an integer indicating the charge of the complex. Generally, c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge. For a number of osmium complexes, c is +2 or +3.

X represents counter ion(s). Examples of suitable counter ions include anions, such as halide (e.g., fluoride, chloride, bromide or iodide), sulfate, phosphate, hexafluorophosphate, and tetrafluoroborate, and cations (preferably, monovalent cations), such as lithium, sodium, potassium, tetralkylammonium, and ammonium. Preferably, X is a halide, such as chloride. The counter ions represented by X are not necessarily all the same.

d represents the number of counter ions and is typically from 1 to 5.

L_1 , L_2 , L_3 and L_4 are ligands attached to the transition metal via a coordinative bond. L_1 , L_2 , L_3 and L_4 can be monodentate ligands or, in any combination, bi-, ter-, or tetradentate ligands. For example, L_1 , L_2 , L_3 and L_4 can combine to form two bidentate ligands such as, for example, two ligands selected from the group of substituted and unsubstituted 2,2'-biimidazoles, 2-(2-pyridyl)imidazoles, and 2,2'-bipyridines

Examples of other L_1 , L_2 , L_3 and L_4 combinations of the transition metal complex include:

- (A) L_1 is a monodentate ligand and L_2 , L_3 and L_4 in combination form a terdentate ligand;
- (B) L_1 and L_2 in combination are a bidentate ligand, and L_3 and L_4 are the same or different monodentate ligands;

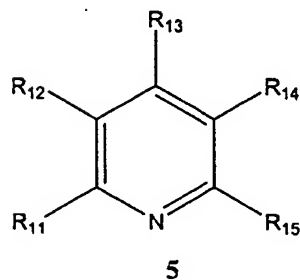
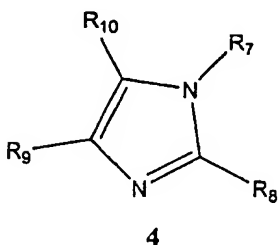
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(C) L_1 and L_2 in combination, and L_3 and L_4 in combination form two independent bidentate ligands which can be the same or different; and

(D) L_1 , L_2 , L_3 and L_4 in combination form a tetradentate ligand.

Examples of suitable monodentate ligands include, but are not limited to, -F,

- 5 -Cl, -Br, -I, -CN, -SCN, -OH, H_2O , NH_3 , alkylamine, dialkylamine, trialkylamine, alkoxy or heterocyclic compounds. The alkyl or aryl portions of any of the ligands are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. Any alkyl portions of the monodentate ligands generally contain 1 to 12
- 10 carbons. More typically, the alkyl portions contain 1 to 6 carbons. In other embodiments, the monodentate ligands are heterocyclic compounds containing at least one nitrogen, oxygen, or sulfur atom. Examples of suitable heterocyclic monodentate ligands include imidazole, pyrazole, oxazole, thiazole, pyridine, pyrazine and derivatives thereof. Suitable heterocyclic monodentate ligands include
- 15 substituted and unsubstituted imidazole and substituted and unsubstituted pyridine having the following general formulas 4 and 5, respectively:



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With regard to formula 4, R₇ is generally a substituted or unsubstituted alkyl, alkenyl, or aryl group. Typically, R₇ is a substituted or unsubstituted C1 to C12 alkyl or alkenyl. The substitution of inner coordination sphere chloride anions by imidazoles does not typically cause a large shift in the redox potential in the oxidizing direction, differing in this respect from substitution by pyridines, which typically results in a large shift in the redox potential in the oxidizing direction.

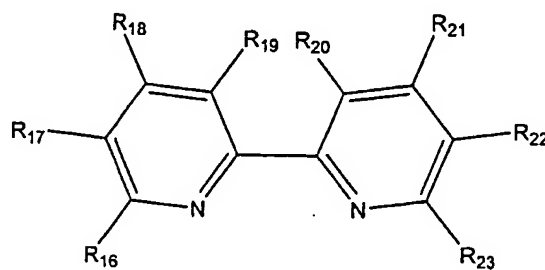
R₈, R₉ and R₁₀ are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, aryl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl. Alternatively, R₉ and R₁₀, in combination, form a fused 5 or 6-membered ring that is saturated or unsaturated. The alkyl portions of the substituents generally contain 1 to 12 carbons and typically contain 1 to 6 carbon atoms. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. In some embodiments, R₈, R₉ and R₁₀ are -H or substituted or unsubstituted alkyl. Preferably, R₈, R₉ and R₁₀ are -H.

With regard to Formula 5, R₁₁, R₁₂, R₁₃, R₁₄ and R₁₅ are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except for aryl portions), alkoxy, alkylthio, aryl, or a reactive group. Generally, R₁₁, R₁₂, R₁₃, R₁₄ and R₁₅ are -H, methyl, C1-C2 alkoxy, C1-C2 alkylamino, C2-C4 dialkylamino, or a C1-C6 lower alkyl substituted with a reactive group.

One example includes R₁₁ and R₁₅ as -H, R₁₂ and R₁₄ as the same and -H or methyl, and R₁₃ as -H, C1 to C12 alkoxy, -NH₂, C1 to C12 alkylamino, C2 to C24 dialkylamino, hydrazino, C1 to C12 alkylhydrazino, hydroxylamino, C1 to C12 alkoxyamino, C1 to C12 alkylthio, or C1 to C12 alkyl. The alkyl or aryl portions of

any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group.

Examples of suitable bidentate ligands include, but are not limited to, amino acids, oxalic acid, acetylacetone, diaminoalkanes, ortho-diaminoarenes, 2,2'-biimidazole, 2,2'-bioxazole, 2,2'-bithiazole, 2-(2-pyridyl)imidazole, and 2,2'-bipyridine and derivatives thereof. Particularly suitable bidentate ligands for redox mediators include substituted and unsubstituted 2,2'-biimidazole, 2-(2-pyridyl)imidazole and 2,2'-bipyridine. The substituted 2,2' biimidazole and 2-(2-pyridyl)imidazole ligands can have the same substitution patterns described above for the other 2,2' -biimidazole and 2-(2-pyridyl)imidazole ligand. A 2,2'-bipyridine ligand has the following general formula 6:



6

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R_{16} , R_{17} , R_{18} , R_{19} , R_{20} , R_{21} , R_{22} and R_{23} are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, aryl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, or alkyl. Typically, the alkyl and alkoxy portions are C1 to C12. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group.

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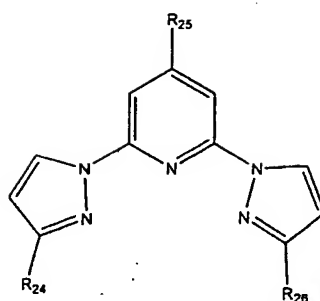
Specific examples of suitable combinations of R_{16} , R_{17} , R_{18} , R_{19} , R_{20} , R_{21} , R_{22} and R_{23} include R_{16} and R_{23} as H or methyl; R_{17} and R_{22} as the same and -H or

methyl; and R_{19} and R_{20} as the same and -H or methyl. An alternative combination is where one or more adjacent pairs of substituents R_{16} and R_{17} , on the one hand, and R_{22} and R_{23} , on the other hand, independently form a saturated or unsaturated 5- or 6-membered ring. Another combination includes R_{19} and R_{20} forming a saturated or
5 unsaturated five or six membered ring.

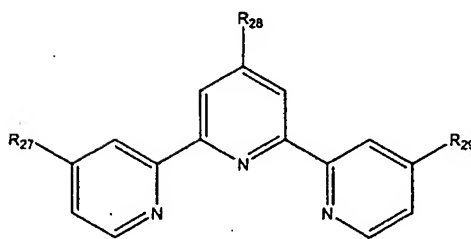
Another combination includes R_{16} , R_{17} , R_{19} , R_{20} , R_{22} and R_{23} as the same and -H and R_{18} and R_{21} as independently -H, alkoxy, -NH₂, alkylamino, dialkylamino, alkylthio, alkenyl, or alkyl. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino,
10 trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. As an example, R_{18} and R_{21} can be the same or different and are -H, C1-C6 alkyl, C1-C6 amino, C1 to C12 alkylamino, C2 to C12 dialkylamino, C1 to C12 alkylthio, or C1 to C12 alkoxy, the alkyl portions of any of the substituents are optionally substituted by a -F, -Cl, -Br, -I, aryl, C2 to C12 dialkylamino, C3 to C18
15 trialkylammonium, C1 to C6 alkoxy, C1 to C6 alkylthio or a reactive group.

Examples of suitable terdentate ligands include, but are not limited to, diethylenetriamine, 2,2',2''-terpyridine, 2,6-bis(N-pyrazolyl)pyridine, and derivatives of these compounds. 2,2',2''-terpyridine and 2,6-bis(N-pyrazolyl)pyridine have the following general formulas 7 and 8 respectively:

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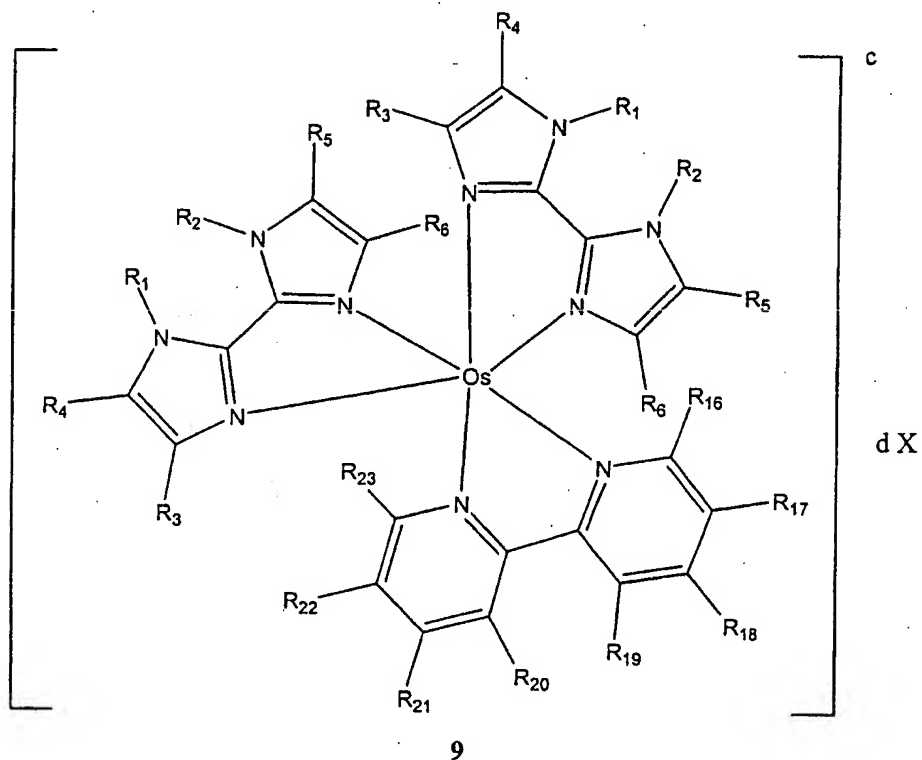
With regard to formula 7, R_{24} , R_{25} and R_{26} are independently -H or substituted or unsubstituted C1 to C12 alkyl. Typically, R_{24} , R_{25} and R_{26} are -H or methyl and, in some embodiments, R_{24} and R_{26} are the same and are -H. Other substituents at these or other positions of the compounds of formulas 7 and 8 can be added.

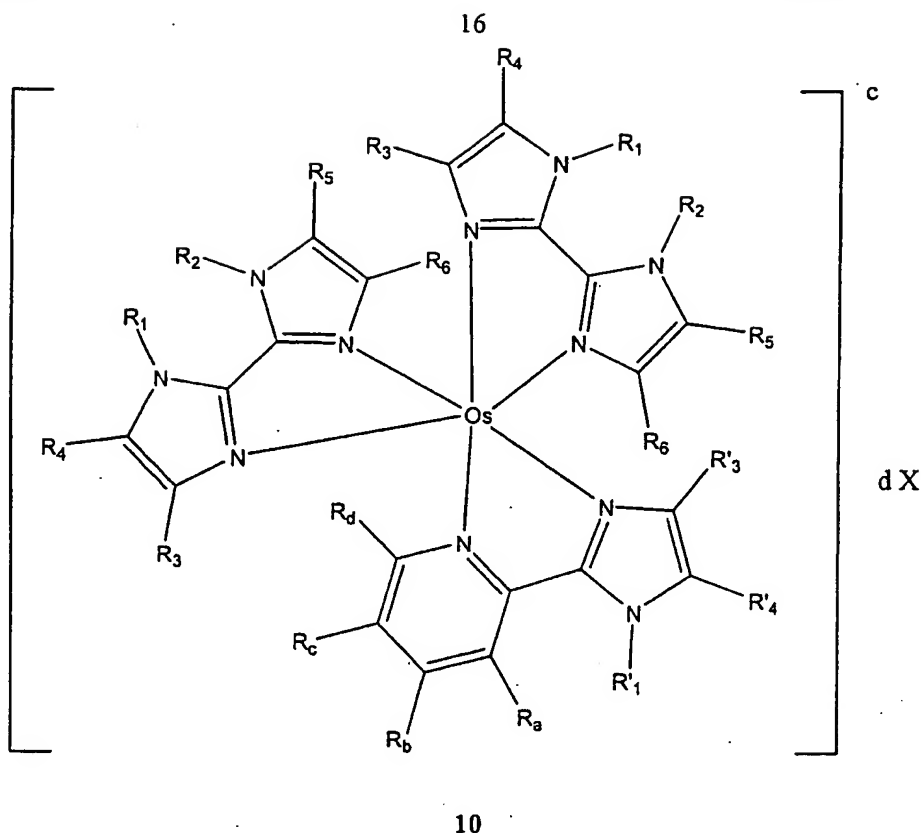
With regard to formula 8, R_{27} , R_{28} and R_{29} are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, -OH, alkoxy, -NH₂, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl. The alkyl or aryl portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group. Typically, the alkyl and alkoxy groups are C1 to C12 and, in some embodiments, R_{27} and R_{29} are the same and are -H.

Examples of suitable tetradentate ligands include, but are not limited to, triethylenetriamine, ethylenediaminediacetic acid, tetraaza macrocycles and similar compounds as well as derivatives thereof.

15

Examples of suitable transition metal complexes are illustrated using
Formula 9 and 10:





With regard to transition metal complexes of formula 9, the metal osmium is
 5 complexed to two substituted 2,2'-biimidazole ligands and one substituted or
 unsubstituted 2,2'-bipyridine ligand. $R_1, R_2, R_3, R_4, R_5, R_6, R_{16}, R_{17}, R_{18}, R_{19}, R_{20},$
 $R_{21}, R_{22}, R_{23}, c, d,$ and X are the same as described above.

In one embodiment, R_1 and R_2 are methyl; $R_3, R_4, R_5, R_6, R_{16}, R_{17}, R_{19}, R_{20},$
 R_{22} and R_{23} are -H; and R_{18} and R_{21} are the same and are -H, methyl, or methoxy.
 10 Preferably, R_{18} and R_{21} are methyl or methoxy.

In another embodiment, R_1 and R_2 are methyl; $R_3, R_4, R_5, R_6, R_{16}, R_{17}, R_{18},$
 R_{19}, R_{20}, R_{22} and R_{23} are -H; and R_{21} is halo, C1 to C12 alkoxy, C1 to C12
 alkylamino, or C2 to C24 dialkylamino. The alkyl or aryl portions of any of the
 substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino,
 15 trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive
 group. For example, R_{21} is a C1 to C12 alkylamino or C2 to C24 dialkylamino, the
 alkyl portion(s) of which are substituted with a reactive group, such as a carboxylic

acid, activated ester, or amine. Typically, the alkylamino group has 1 to 6 carbon atoms and the dialkylamino group has 2 to 8 carbon atoms.

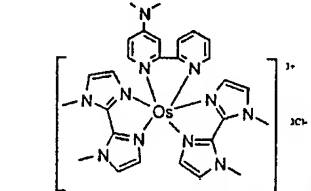
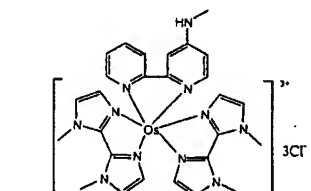
With regard to transition metal complexes of formula 10, the metal osmium is complexed to two substituted 2,2'-biimidazole ligands and one substituted or
 5 unsubstituted 2-(2-pyridyl)imidazole ligand. $R_1, R_2, R_3, R_4, R_5, R_6, R'_1, R'_3, R'_4, R_5, R_6, R_c, R_d$, c, d, and X are the same as described above.

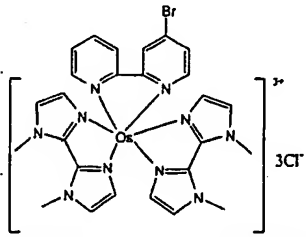
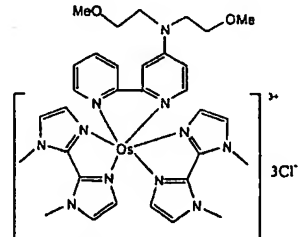
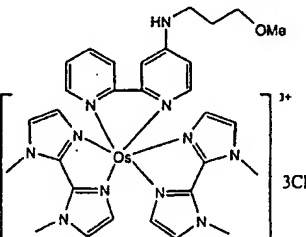
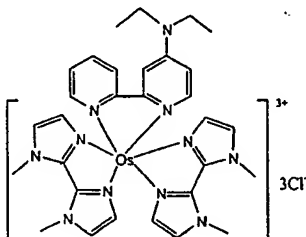
In one embodiment, R_1 and R_2 are methyl; $R_3, R_4, R_5, R_6, R'_3, R'_4$ and R_d are independently -H or methyl; R_c and R_e are the same and are -H; and R_b is C1 to C12 alkoxy, C1 to C12 alkylamino, or C2 to C24 dialkylamino. The alkyl or aryl
 10 portions of any of the substituents are optionally substituted by -F, -Cl, -Br, -I, alkylamino, dialkylamino, trialkylammonium (except on aryl portions), alkoxy, alkylthio, aryl, or a reactive group.

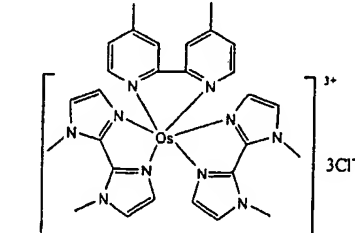
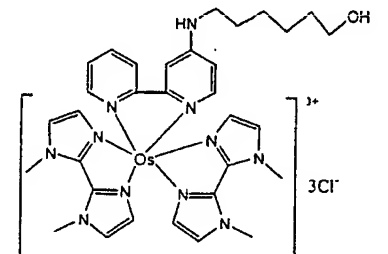
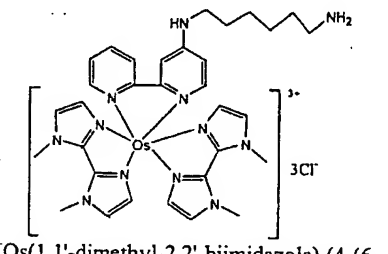
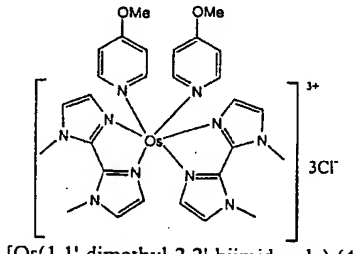
A list of specific examples of preferred transition metal complexes with respective redox potentials is shown in Table 1.

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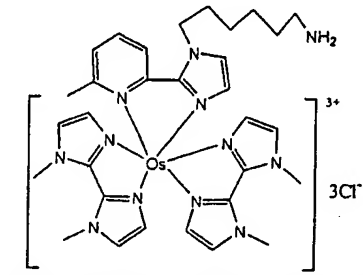
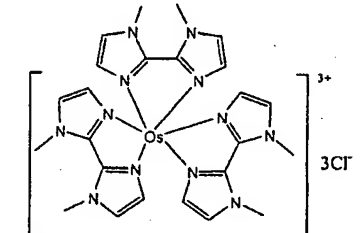
Table 1. Redox Potentials of Selected Transition Metal Complexes

Complex	Structure	$E_{1/2}(\text{vs Ag/AgCl})/\text{mV}^*$
<u>I</u>	 $[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-dimethylamino-2,2'-bipyridine})]\text{Cl}_3$	-110
<u>II</u>	 $[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-methylamino-2,2'-bipyridine})]\text{Cl}_3$	-100

<u>III</u>	 <p>[Os(1,1'-dimethyl-2,2'-biimidazole)₂(4-bromo-2,2'-bipyridine)]Cl₃</p>	128
<u>IV</u>	 <p>[Os(1,1'-dimethyl-2,2'-biimidazole)₂(4-di(2-methoxyethyl)amino-2,2'-bipyridine)]Cl₃</p>	-86
<u>V</u>	 <p>[Os(1,1'-dimethyl-2,2'-biimidazole)₂(4-(3-methoxypropyl)amino-2,2'-bipyridine)]Cl₃</p>	-97
<u>VI</u>	 <p>[Os(1,1'-dimethyl-2,2'-biimidazole)₂(4-diethylamino-2,2'-bipyridine)]Cl₃</p>	-120

<u>VII</u>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4,4'\text{-dimethyl-2,2'-bipyridine})]\text{Cl}_3$</p>	32
<u>VIII</u>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-(6-hydroxyhexyl)amino-2,2'-bipyridine})]\text{Cl}_3$</p>	-100
<u>IX</u>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-(6-aminoethyl)amino-2,2'-bipyridine})]\text{Cl}_3$</p>	-93
<u>X</u>	 <p>$[\text{Os}(1,1'\text{-dimethyl-2,2'-biimidazole})_2(4\text{-methoxypyridine})_2]\text{Cl}_3$</p>	-125

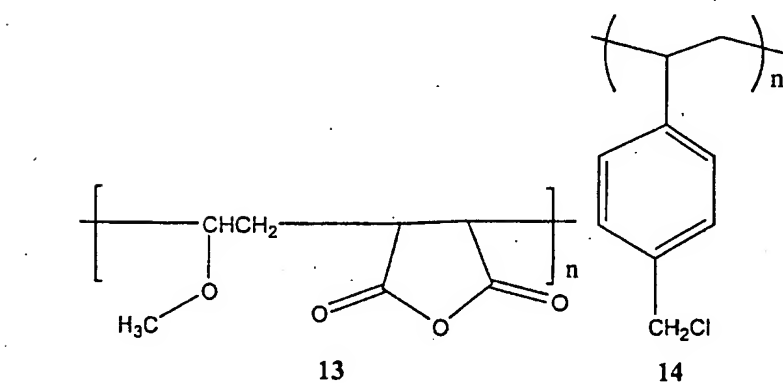
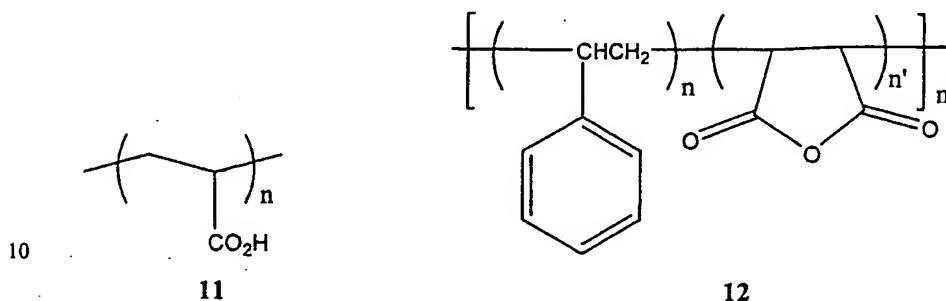
<u>XI</u>	<p>[Os(1,1'-dimethyl-2,2'-biimidazole)₂(4-(N-(4-carboxy)piperidino)-2,2'-bipyridine)]Cl₃</p>	-60
<u>XII</u>	<p>[Os(1,1'-dimethyl-2,2'-biimidazole)₂(1-methyl-2-(2-pyridyl)imidazole)]Cl₃</p>	-74
<u>XIII</u>	<p>[Os(1,1'-dimethyl-2,2'-biimidazole)₂(1-methyl-2-(6-methylpyrid-2-yl)imidazole)]Cl₃</p>	-97

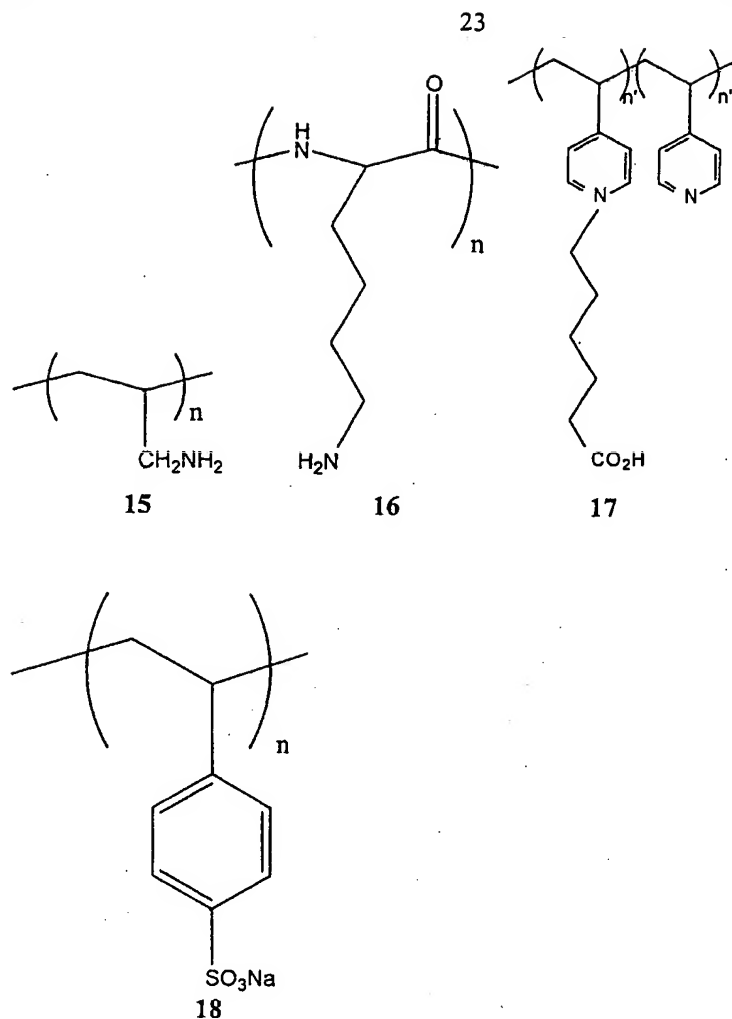
<p><u>XIV</u></p>	 <p>[Os(1,1'-dimethyl-2,2'-biimidazole)₂(1-(6-aminoheptyl)-2-(6-methylpyrid-2-yl)imidazole)₂]³⁺ 3Cl⁻</p>	<p>-81</p>
<p><u>XV</u></p>	 <p>[Os(3,3'-dimethyl-2,2'-biimidazole)₂]³⁺ 3Cl⁻</p>	<p>-230</p>
<p>* Redox potentials were estimated by averaging the positions of the reduction wave peaks and the oxidation wave peaks of cyclic voltammograms (CVs) obtained in pH 7 PBS buffer with a glassy carbon working electrode, a graphite counter electrode and a standard Ag/AgCl reference electrode at a sweep rate of 50 mV/s.</p>		

The transition metal complexes of Formula 1 also include transition metal complexes that are coupled to a polymeric backbone through one or more of L₁, L₂, L₃, and L₄. Additional examples of suitable transition metal complexes are described in U.S. Patent Application Serial No. _____, entitled "Polymeric Transition Metal Complexes and Uses Thereof", filed on even date herewith, Attorney Docket No. 12008.41US02, incorporated herein by reference. In some embodiments, the polymeric backbone has functional groups that act as ligands of the transition metal complex. Such polymeric backbones include, for example, poly(4-vinylpyridine) and poly(N-vinylimidazole) in which the pyridine and imidazole groups, respectively, can act as monodentate ligands of the transition metal complex. In other embodiments, the transition metal complex can be the

reaction product between a reactive group on a precursor polymer and a reactive group on a ligand of a precursor transition metal complex (such as complex of Formula 1 where one of L , L_1 , L_2 , L_3 , and L_4 includes a reactive group, as described above). Suitable precursor polymers include, for example, poly(acrylic acid)

- 5 (Formula 11), styrene/maleic anhydride copolymer (Formula 12), methylvinylether/maleic anhydride copolymer (GANTREZ polymer) (Formula 13), poly(vinylbenzylchloride) (Formula 14), poly(allylamine) (Formula 15), polylysine (Formula 16), carboxy-poly(vinylpyridine) (Formula 17), and poly(sodium 4-styrene sulfonate) (Formula 18).





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Alternatively, the transition metal complex can have reactive group(s) for immobilization or conjugation of the complexes to other substrates or carriers, examples of which include, but are not limited to, macromolecules (e.g., enzymes) and surfaces (e.g., electrode surfaces).

- 10 For reactive attachment to polymers, substrates, or other carriers, the transition metal complex precursor includes at least one reactive group that reacts with a reactive group on the polymer, substrate, or carrier. Typically, covalent bonds are formed between the two reactive groups to generate a linkage. Examples of such linkages are provided in Table 2, below. Generally, one of the reactive
- 15 groups is an electrophile and the other reactive group is a nucleophile.

Table 2: Examples of Reactive Group Linkages

First Reactive Group	Second Reactive Group	Resulting Linkage
Activated ester*	Amine	Carboxamide
Acrylamide	Thiol	Thioether
Acyl azide	Amine	Carboxamide
Acyl halide	Amine	Carboxamide
Carboxylic acid	Amine	Carboxamide
Aldehyde or ketone	Hydrazine	Hydrazone
Aldehyde or ketone	Hydroxyamine	Oxime
Alkyl halide	Amine	Alkylamine
Alkyl halide	Carboxylic acid	Carboxylic ester
Alkyl halide	Imidazole	Imidazolium
Alkyl halide	Pyridine	Pyridinium
Alkyl halide	Alcohol/phenol	Ether
Alkyl halide	Thiol	Thioether
Alkyl sulfonate	Thiol	Thioether
Alkyl sulfonate	Pyridine	Pyridinium
Alkyl sulfonate	Imidazole	Imidazolium
Alkyl sulfonate	Alcohol/phenol	Ether
Anhydride	Alcohol/phenol	Ester
Anhydride	Amine	Carboxamide
Aziridine	Thiol	Thioether
Aziridine	Amine	Alkylamine
Aziridine	Pyridine	Pyridinium
Epoxide	Thiol	Thioether
Epoxide	Amine	Alkylamine
Epoxide	Pyridine	Pyridinium
Halotriazine	Amine	Aminotriazine
Halotriazine	Alcohol	Triazinyl ether
Imido ester	Amine	Amidine
Isocyanate	Amine	Urea
Isocyanate	Alcohol	Urethane
Isothiocyanate	Amine	Thiourea
Maleimide	Thiol	Thioether
Sulfonyl halide	Amine	Sulfonamide
* Activated esters, as understood in the art, generally include esters of succinimidyl, benzotriazolyl, or aryl substituted by electron-withdrawing groups such as sulfo, nitro, cyano, or halo; or carboxylic acids activated by carbodiimides.		

Transition metal complexes of the present invention can be soluble in water
 5 or other aqueous solutions, or in organic solvents. In general, the transition metal
 complexes can be made soluble in either aqueous or organic solvents by having an

appropriate counter ion or ions, X. For example, transition metal complexes with small counter anions, such as F⁻, Cl⁻, and Br⁻, tend to be water soluble. On the other hand, transition metal complexes with bulky counter anions, such as I⁻, BF₄⁻ and PF₆⁻, tend to be soluble in organic solvents. Preferably, the solubility of transition metal complexes of the present invention is greater than about 0.1 M (moles/liter) at 25 °C for a desired solvent.

The transition metal complexes discussed above are useful as redox mediators in electrochemical sensors for the detection of analytes in bio-fluids. The use of transition metal complexes as redox mediators is described, for example, in U.S. Patents Nos. 5,262,035, 5,262,305, 5,320,725, 5,365,786, 5,593,852, 5,665,222, 5,972,199, and 6,143,164 and U.S. Patent Applications Serial Nos. 09/034,372, 09/070,677, 09/295,962, and 09/434,026, all of which are herein incorporated by reference. The transition metal complexes described herein can typically be used in place of those discussed in the references listed above. The transition metal complexes that include a polymeric backbone and are redox mediators can also be referred to as "redox polymers".

In general, the redox mediator is disposed on or in proximity to (e.g., in a solution surrounding) a working electrode. The redox mediator transfers electrons between the working electrode and an analyte. In some preferred embodiments, an enzyme is also included to facilitate the transfer. For example, the redox mediator transfers electrons between the working electrode and glucose (typically via an enzyme) in an enzyme-catalyzed reaction of glucose. Redox polymers are particularly useful for forming non-leachable coatings on the working electrode. These can be formed, for example, by crosslinking the redox polymer on the working electrode, or by crosslinking the redox polymer and the enzyme on the working electrode

Transition metal complexes can enable accurate, reproducible and quick or continuous assays. Transition metal complex redox mediators accept electrons from, or transfer electrons to, enzymes or analytes at a high rate and also exchange electrons rapidly with an electrode. Typically, the rate of self exchange, the process in which a reduced redox mediator transfers an electron to an oxidized redox mediator, is rapid. At a defined redox mediator concentration, this provides for

more rapid transport of electrons between the enzyme (or analyte) and electrode, and thereby shortens the response time of the sensor. Additionally, the novel transition metal complex redox mediators are typically stable under ambient light and at the temperatures encountered in use, storage and transportation. Preferably, the transition metal complex redox mediators do not undergo chemical change, other than oxidation and reduction, in the period of use or under the conditions of storage, though the redox mediators can be designed to be activated by reacting, for example, with water or the analyte.

The transition metal complex can be used as a redox mediator in combination with a redox enzyme to electrooxidize or electroreduce the analyte or a compound derived of the analyte, for example by hydrolysis of the analyte. The redox potentials of the redox mediators are generally more positive (i.e. more oxidizing) than the redox potentials of the redox enzymes when the analyte is electrooxidized and more negative when the analyte is electroreduced. For example, the redox potentials of the preferred transition metal complex redox mediators used for electrooxidizing glucose with glucose oxidase or PQQ-glucose dehydrogenase as enzyme is between about - 200 mV and + 200 mV versus a Ag/AgCl reference electrode, and the most preferred mediators have redox potentials between about - 100 mV and about +100 mV versus a Ag/AgCl reference electrode

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Crosslinking in Transition Metal Complex Polymers

Electron transport involves an exchange of electrons between segments of the redox polymers (e.g., one or more transition metal complexes coupled to a polymeric backbone, as described above) in a crosslinked film disposed on an electrode. The transition metal complex can be bound to the polymer backbone though covalent, coordinative or ionic bonds, where covalent and coordinative binding are preferred. Electron exchange occurs, for example, through the collision of different segments of the crosslinked redox polymer. Electrons transported through the redox polymer can originate from, for example, electrooxidation or electroreduction of an enzymatic substrate, such as, for example, the oxidation of glucose by glucose oxidase.

30

The degree of crosslinking of the redox polymer can influence the transport of electrons or ions and thereby the rates of the electrochemical reactions. Excessive crosslinking of the polymer can reduce the mobility of the segments of the redox polymer. A reduction in segment mobility can slow the diffusion of electrons or ions through the redox polymer film. A reduction in the diffusivity of electrons, for example, can require a concomitant reduction in the thickness of the film on the electrode where electrons or electron vacancies are collected or delivered. The degree of crosslinking in a redox polymer film can thus affect the transport of electrons from, for example, an enzyme to the transition metal redox centers of the redox polymer such as, for example, $\text{Os}^{2+/3+}$ metal redox centers; between redox centers of the redox polymer; and from these transition metal redox centers to the electrode.

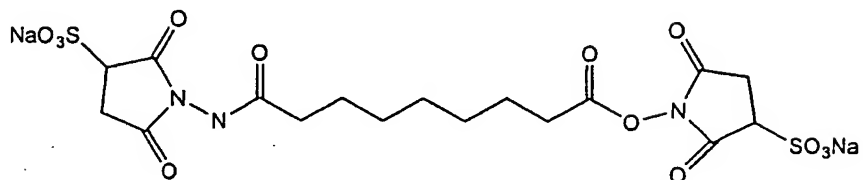
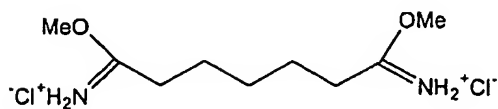
Inadequate crosslinking of a redox polymer can result in excessive swelling of the redox polymer film and to the leaching of the components of the redox polymer film. Excessive swelling can also result in the migration of the swollen polymer into the analyzed solution, in the softening of the redox polymer film, in the film's susceptibility to removal by shear, or any combination of these effects.

Crosslinking can decrease the leaching of film components and can improve the mechanical stability of the film under shear stress. For example, as disclosed in Binyamin, G. and Heller, A; *Stabilization of Wired Glucose Oxidase Anodes Rotating at 1000 rpm at 37 °C*; Journal of the Electrochemical Society, 146(8), 2965-2967, 1999, herein incorporated by reference, replacing a difunctional crosslinker, such as polyethylene glycol diglycidyl ether, with a trifunctional crosslinker such as *N,N*-diglycidyl-4-glycidyl-oxyaniline, for example, can reduce leaching and shear problems associated with inadequate crosslinking.

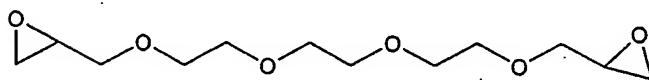
Examples of other bifunctional, trifunctional and tetrafunctional crosslinkers are listed below:

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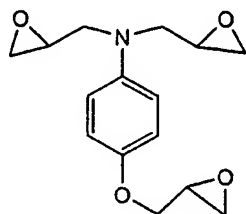
Amine-reactive Bifunctional Crosslinkers



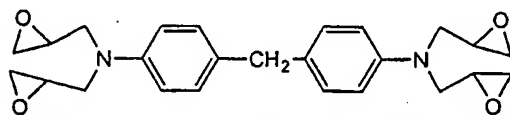
Pyridine- or Imidazole-reactive Bifunctional Crosslinkers



Pyridine- or Imidazole-reactive trifunctional Crosslinker



Pyridine- or Imidazole-reactive Tetrafunctional Crosslinkers



Alternatively, the number of crosslinking sites can be increased by reducing the number of transition metal complexes attached to the polymeric backbone, thus making more polymer pendant groups available for crosslinking. One important

advantage of at least some of the redox polymers is the increased mobility of the pendant transition metal complexes, resulting from the flexibility of the pendant groups. As a result, in at least some embodiments, fewer transition metal complexes per polymer backbone are needed to achieve a desired level of diffusivity of
5 electrons and current density of analyte electrooxidation or electroreduction.

Coordination in Transition Metal Complex Polymers

Transition metal complexes can be directly or indirectly attached to a polymeric backbone, depending on the availability and nature of the reactive groups on the complex and the polymeric backbone. For example, the pyridine groups in
10 poly(4-vinylpyridine) or the imidazole groups in poly(N-vinylimidazole) are capable of acting as monodentate ligands and thus can be attached to a metal center directly. Alternatively, the pyridine groups in poly(4-vinylpyridine) or the imidazole groups in poly(N-vinylimidazole) can be quaternized with a substituted alkyl moiety having
15 a suitable reactive group, such as a carboxylate function, that can be activated to form a covalent bond with a reactive group, such as an amine, of the transition metal complex. (See Table 2 for a list of other examples of reactive groups.)

Redox centers such as, for example, $\text{Os}^{2+/3+}$ can be coordinated with five heterocyclic nitrogens and an additional ligand such as, for example, a chloride
20 anion. An example of such a coordination complex includes two bipyridine ligands which form stable coordinative bonds, the pyridine of poly(4-vinylpyridine) which forms a weaker coordinative bond, and a chloride anion which forms the least stable coordinative bond.

Alternatively, redox centers, such as $\text{Os}^{2+/3+}$, can be coordinated with six
25 heterocyclic nitrogen atoms in its inner coordination sphere. The six coordinating atoms are preferably paired in the ligands, for example, each ligand is composed of at least two rings. Pairing of the coordinating atoms can influence the potential of an electrode used in conjunction with redox polymers of the present invention.

Typically, for analysis of glucose, the potential at which the working
30 electrode, coated with the redox polymer, is poised is negative of about +250 mV vs. SCE (standard calomel electrode). Preferably, the electrode is poised negative of about +150 mV vs. SCE. Poising the electrode at these potentials reduces the

interfering electrooxidation of constituents of biological solutions such as, for example, urate, ascorbate and acetaminophen. The potential can be modified by altering the ligand structure of the complex.

The redox potential of a redox polymer, as described herein, is related to the potential at which the electrode is poised. Selection of a redox polymer with a desired redox potential allows tuning of the potential at which the electrode is best poised. The redox potentials of a number of the redox polymers described herein are negative of about +150 mV vs. SCE and can be negative of about +50 mV vs. SCE to allow the poisoning of the electrode potentials negative of about +250 mV vs. SCE and preferably negative of about +150 mV vs. SCE.

The strength of the coordination bond can influence the potential of the redox centers in the redox polymers. Typically, the stronger the coordinative bond, the more positive the redox potential. A shift in the potential of a redox center resulting from a change in the coordination sphere of the transition metal can produce a labile transition metal complex. For example, when the redox potential of an $\text{Os}^{2+/3+}$ complex is downshifted by changing the coordination sphere, the complex becomes labile. Such a labile transition metal complex may be undesirable when fashioning a metal complex polymer for use as a redox mediator and can be avoided through the use of weakly coordinating multidentate or chelating heterocyclics as ligands.

Electrode Interference

Transition metal complexes used as redox mediators in electrodes can be affected by the presence of transition metals in the analyzed sample including, for example, Fe^{3+} or Zn^{2+} . The addition of a transition metal cation to a buffer used to test an electrode results in a decline in the current produced. The degree of current decline depends on the presence of anions in the buffer which precipitate the transition metal cations. The lesser the residual concentration of transition metal cations in the sample solution, the more stable the current. Anions which aid in the precipitation of transition metal cations include, for example, phosphate. It has been found that a decline in current upon the addition of transition metal cations is most pronounced in non-phosphate buffers. If an electrode is transferred from a buffer

containing a transition metal cation to a buffer substantially free of the transition metal cation, the original current is restored.

The decline in current is thought to be due to additional crosslinking of a pyridine-containing polymer backbone produced by the transition metal cations.

5 The transition metal cations can coordinate nitrogen atoms of different chains and chain segments of the polymers. Coordinative crosslinking of nitrogen atoms of different chain segments by transition metal cations can reduce the diffusivity of electrons.

Serum and other physiological fluids contain traces of transition metal ions, which can diffuse into the films of electrodes made with the redox polymers of the present invention, lowering the diffusivity of electrons and thereby the highest current reached at high analyte concentration. In addition, transition metal ions like iron and copper can bind to proteins of enzymes and to the reaction centers or channels of enzymes, reducing their turnover rate. The resulting decrease in sensitivity can be remedied through the use of anions which complex with interfering transition metal ions, for example, in a buffer employed during the production of the transition metal complex. A non-cyclic polyphosphate such as, for example, pyrophosphate or triphosphate, can be used. For example, sodium or potassium non-cyclic polyphosphate buffers can be used to exchange phosphate anions for those anions in the transition metal complex which do not precipitate transition metal ions. The use of linear phosphates can alleviate the decrease in sensitivity by forming strong complexes with the damaging transition metal ions, assuring that their activity will be low. Other complexing agents can also be used as long as they are not electrooxidized or electroreduced at the potential at which the electrode is poised.

Enzyme Damage and its Alleviation

Glucose oxidase is a flavoprotein enzyme that catalyzes the oxidation by dioxygen of D-glucose to D-glucono-1,5-lactone and hydrogen peroxide. Reduced transition metal cations such as, for example, Fe^{2+} , and some transition metal complexes, can react with hydrogen peroxide. These reactions form destructive OH radicals and the corresponding oxidized cations. The presence of these newly

formed transition metal cations can inhibit the enzyme and react with the metal complex. Also, the oxidized transition metal cation can be reduced by the FADH_2 centers of an enzyme, or by the transition metal complex.

Inhibition of the active site of an enzyme or a transition metal complex by a transition metal cation, as well as damaging reactions with OH radicals can be alleviated, thus increasing the sensitivity and functionality of the electrodes by incorporating non-cyclic polyphosphates, as discussed above. Because the polyphosphate/metal cation complex typically has a high (oxidizing) redox potential, its rate of oxidation by hydrogen peroxide is usually slow. Alternatively, an enzyme such as, for example, catalase, can be employed to degrade hydrogen peroxide.

EXAMPLES

Unless indicated otherwise, all of the chemical reagents are available from Aldrich Chemical Co. (Milwaukee, WI) or other sources. Additional examples are provided in U.S. Patent Application Serial No. _____, entitled "Polymeric Transition Metal Complexes and Uses Thereof", filed on even date herewith, Attorney Docket No. 12008.41US02, incorporated herein by reference. For purposes of illustration, the synthesis of several transition metal complex ligands are shown below:

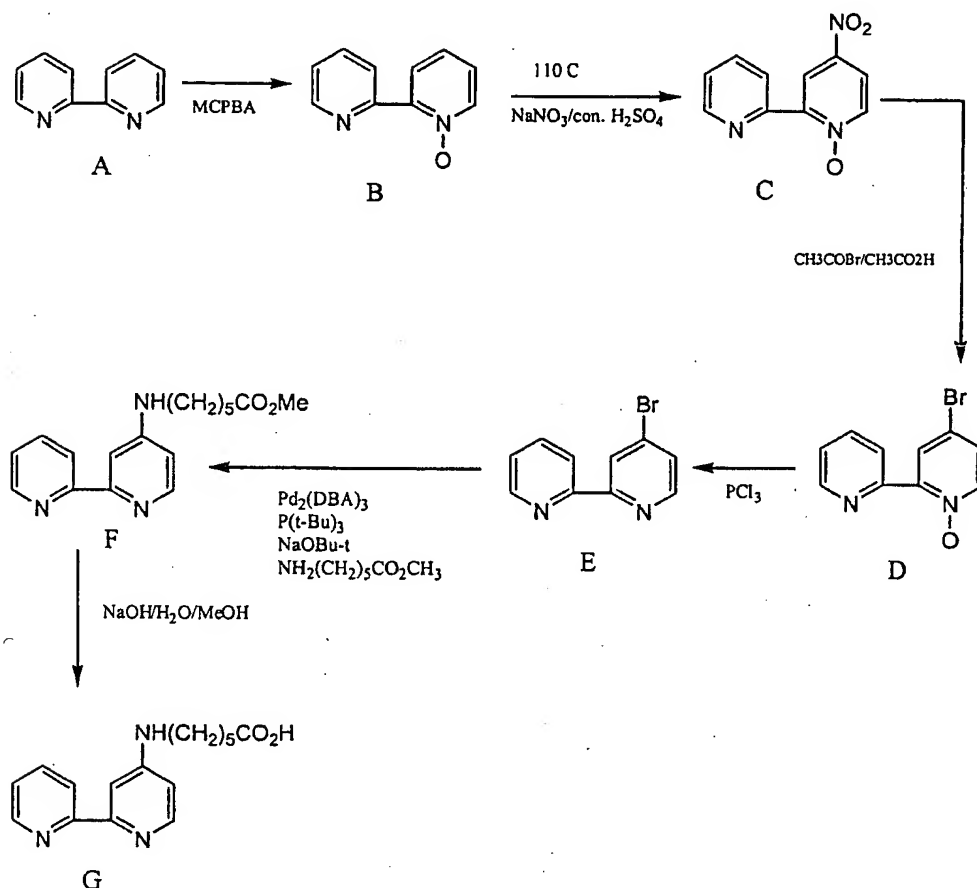
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Example 1

Synthesis of 4-(5-carboxypentyl)amino-2,2'-bipyridyl

This example illustrates how a carboxy reactive group is introduced onto a 2,2'-bipyridyl derivative.

Synthesis of compound D: To compound C (formed from A and B)



according to Wenkert, D.; Woodward, R.B. *J. Org. chem.* **48**, 283(1983)) (5 g) dissolved in 30 mL acetic acid in a 100 mL round bottom flask was added 16 mL acetyl bromide. The yellow mixture was refluxed for 1.5 h and then rotovaporated to dryness. The resulting light yellow solid of **D** was sufficiently pure enough for the next step without further purification. Yield: 95%

Synthesis of compound E: To a stirred suspension of compound **D** in 60 mL CHCl_3 was added 12 mL PCl_3 at room temperature. The mixture was refluxed for 2 h under N_2 and then cooled to room temperature. The reaction mixture was poured into 100 mL ice/water. The aqueous layer was separated and saved. The CHCl_3 layer was extracted three times with H_2O (3x60 mL) and then discarded. The combined aqueous solution was neutralized with NaHCO_3 powder to about pH 7 to

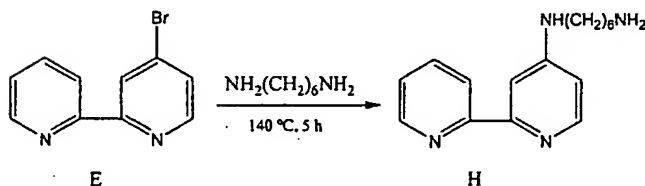
8. The resulting white precipitate was collected by suction filtration, washed with H_2O (30 mL) and then dried under vacuum at 50°C for 24 h. Yield: 85%.

Synthesis of compound F: Compound F was synthesized from compound E (5 g) and 6-aminocaproic acid methyl ester (6g) using the palladium-catalyzed amination method of aryl bromides described by Hartwig et al. (Hartwig, J.F., et al. *J. Org. Chem.* 64, 5575 (1999)). Yield: 90%.

Synthesis of compound G: Compound F (3 g) dissolved in 20 mL MeOH was added to a solution of NaOH (0.6g) in 30 mL H_2O . The resulting solution was stirred at room temperature for 24 h and then neutralized to pH 7 with dilute HCl. The solution was saturated with NaCl and then extracted with CHCl_3 . The CHCl_3 extract was evaporated to dryness and then purified by a silica gel column eluted with 10% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$. Yield: 70%.

Example 2

15 **Synthesis of a 4-((6-Aminohexyl)amino)-2,2'-bipyridine:**



This example illustrates the general synthesis of a 2,2'-bipyridyl with an amine reactive group.

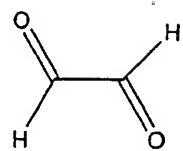
Synthesis of compound H: A mixture of compound E (2.5 g) and 1,6-diaminohexane (15 g) in a 250mL round bottom flask was heated under N_2 at 140°C in an oil bath for 4-5 h. Excess 1,6-diaminohexane was removed by high vacuum distillation at $90\text{--}120^\circ\text{C}$. The product was purified by a silica gel column, eluting with 5% NH_4OH in isopropyl alcohol. Yield: 70%.

Example 3

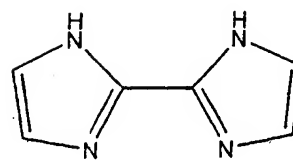
Synthesis of 1,1'-dimethyl-2,2'-biimidazole

This example illustrates the synthesis of 2,2'-biimidazole derivatives.

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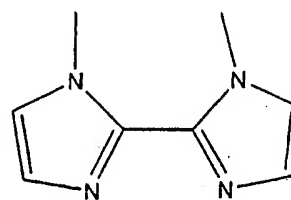
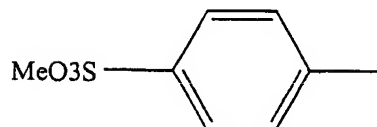
I



J

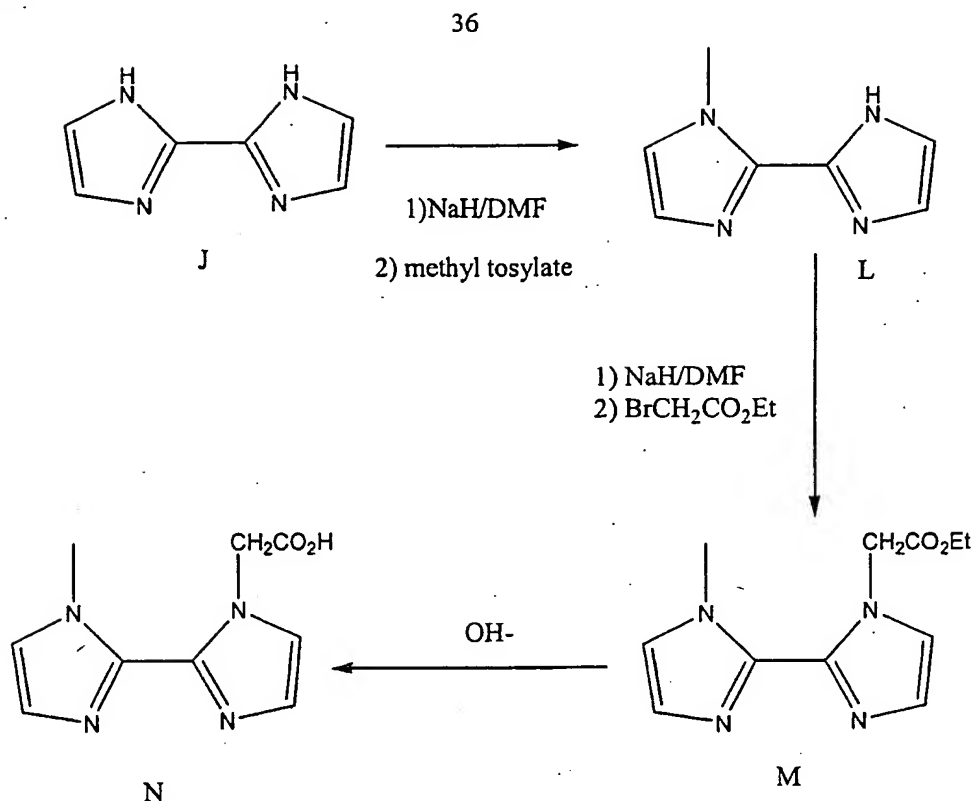
1) NaH/DMF

2)



K

The alkylation step can be carried out stepwise so two different alkyl groups can be introduced. For example:



Synthesis of compound K: To a stirred solution of compound J (formed from I according to Fieselmann, B.F., et al. *Inorg. Chem.* 17, 2078(1978)) (4.6g, 34.3 mmol) in 100 mL dry DMF in a 250 ml round bottom flask cooled in an ice/water bath was added in portions NaH(60% in mineral oil, 2.7 g, 68.6 mmol). After the solution was stirred at 0°C for one more hour under N₂, methyl toluenesulfonate (10.3 mL, 68.6 mmol) was added in small portions using a syringe over 30 min. The stirring of the solution in the ice/water bath was continued for 1 h and then at room temperature for 3 h. The solvent was removed by vacuum distillation. The dark residue was triturated with ether and then suction filtered and dried under vacuum. The product was purified by sublimation. Yield: 80%.

Synthesis of compound L: Compound L was prepared using the method described for the synthesis of compound K except that only one equivalent each of compound J, NaH and methyl toluenesulfonate was used. The product was purified by sublimation.

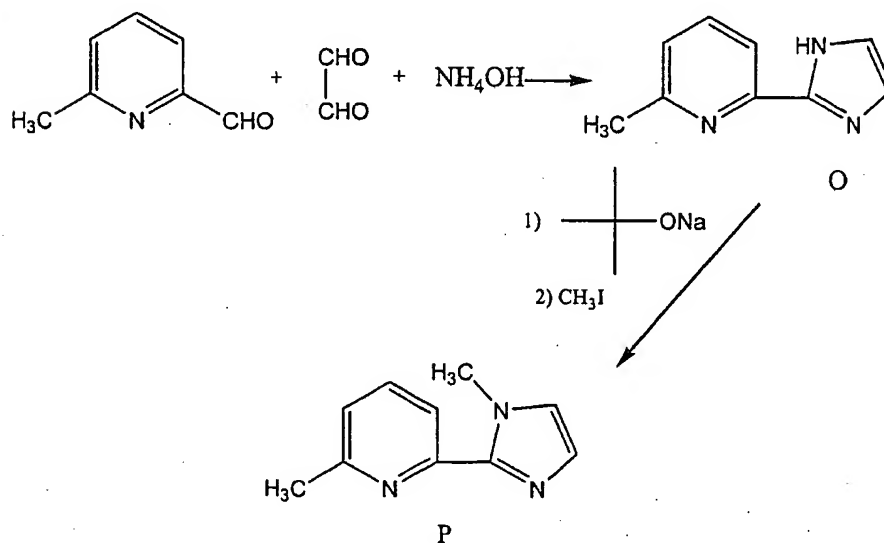
Synthesis of compound M: To a stirred solution of compound L (1g, 6.8 mmol) in 20 mL dry DMF in a 50 mL round bottom flask cooled in an ice/water bath is added in portions NaH (60% in mineral oil, 0.27 g, 6.8 mmol). After the solution is stirred at 0°C for one more hour under N₂, ethyl bromoacetate (0.75 mL, 6.8 mmol) is added in small portions via a syringe over 15 min. The stirring of the solution is continued in the ice/water bath for 1 h and then at room temperature for 3 h. The solvent is removed by vacuum distillation. The product is purified by a silica gel column using 10% MeOH/CHCl₃ as the eluent.

Synthesis of Compound N: Compound M (1g) is hydrolyzed using the method described for the synthesis of compound G. The product is purified by a silica gel column using 10% H₂O/CH₃CN as the eluent.

Example 4

Synthesis of 2-(2-Pyridyl)imidazole Heterobidentate Ligands

This example illustrates a general synthesis of heterobidentate ligands containing an imidazole ring.



Synthesis of compound O: A solution of 6-methylpyridine-2-carboxaldehyde (26g, 0.21 mole) and glyoxal (40%, 30 mL) in 50 mL EtOH in a three-necked 250 mL round bottom flask fitted with a thermometer and an addition

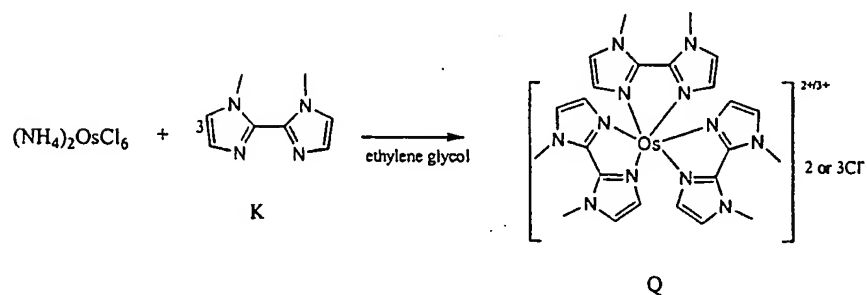
funnel was stirred in a NaCl/ice bath. When the solution was cooled to below 5°C, conc. NH₄OH was added dropwise through the addition funnel. The rate of the addition was controlled so that the temperature of the solution was maintained at below 5°C. After the addition, the stirring of the yellow solution was continued in the ice bath for 1 h and then at room temperature overnight. The light yellow crystals were collected by suction filtration and washed with H₂O (20 mL). The crystals were resuspended in H₂O (200 mL) and boiled briefly, followed by suction filtration, to collect the product which was dried under high vacuum. Yield: 35%.

Synthesis of compound P: Sodium t-butoxide (2g, 20.8 mmoles) was added in one portion to a stirred solution of compound O (3g, 18.9 mmoles) in 50 mL dry DMF. After all of the sodium t-butoxide was dissolved, iodomethane (1.3 mL) was added dropwise using a syringe. The stirring of the solution was continued at room temperature for 2 h and then the solution was poured into H₂O (150mL). The product was extracted with EtOAc, and the extract was dried with anhydrous Na₂SO₄ and then evaporated to give crude compound P. The product was purified by separation on a silica gel column using 10% MeOH/CHCl₃ as the eluent. Yield: 70%.

Example 5

Synthesis of Transition Metal Complexes with Multiple Identical Ligands

Transition metal complexes containing multiple identical bidentate or tridentate ligands can be synthesized in one step from a metal halide salt and the ligand. This example illustrates the synthesis of an osmium complex with three identical 2,2'-biimidazole bidentate ligands.



Synthesis of compound Q: Ammonium hexachloroosmate (200 mg, 0.46 mmols) and compound K (221mg, 1.37 mmols) were mixed in 15 mL ethylene glycol in a 100 mL three-necked round bottom flask fitted with a reflux condenser.

The mixture was degassed with N₂ for 15 min and then stirred under N₂ at 200-210 °

- 5 C for 24 hrs. The solvent was removed by high vacuum distillation at 90-100°C. The green colored crude product was dissolved in 15 mL H₂O and stirred in air to be fully oxidized to the dark blue colored Os(III) oxidation state (about 24 h). The product was purified on a LH-20 reverse phase column using H₂O as the eluent. Yield: 50%.

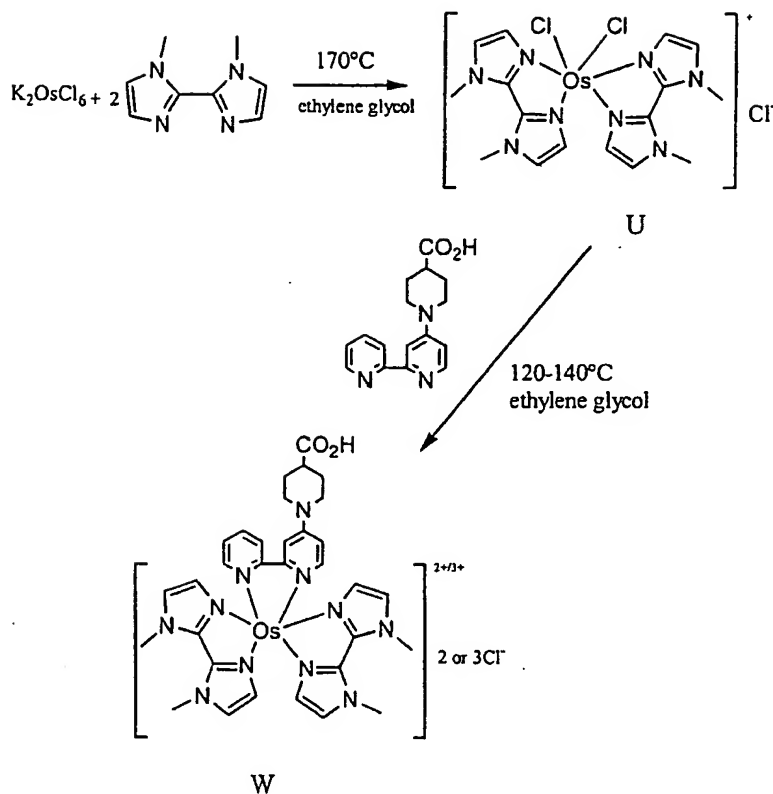
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Example 6

Synthesis of Transition Metal Complexes with Mixed Ligands

Transition metal complexes containing multiple types of ligands can be synthesized stepwise. First, a transition metal complex intermediate that contains

- 15 one desired type of ligand and halide ligand(s), for example, chloride, is synthesized. Then the intermediate is subjected to a ligand substitution reaction to displace the halide ligand(s) with another desired type of ligand. The preparation of the following osmium complex illustrates the general synthetic scheme.



Synthesis of Compound U: Potassium hexachloroosmate (1g, 2.08
 mmoles), compound K (0.67g, 4.16 mmoles) and LiCl (1g, 23.8 mmoles) were
 5 suspended in 40 mL ethylene glycol in a 250 mL three-necked round bottom flask
 fitted with a reflux condenser. The suspension was degassed with N_2 for 15 min and
 then stirred under N_2 at 170°C in an oil bath for 7-8 h, resulting in a dark brown
 solution. The solvent was removed by high vacuum distillation at $90-100^\circ\text{C}$ bath
 temperature. The gummy solid was triturated with acetone twice (2x50mL) and then
 10 with H_2O once (50mL). The product was dried at 50°C under high vacuum for 24 h.

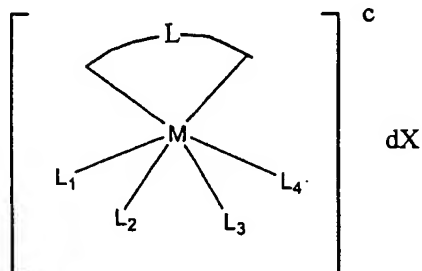
Synthesis of compound W: A suspension of compound U (119mg, 0.192
 mmole) and 4-(4-carboxypiperidino)amino-2,2'-bipyridyl (prepared from compound
 E and ethyl isonipecotatate using the synthetic methods for compounds F and G) was

made in 10 mL ethylene glycol in a 100mL three-necked round bottom flask equipped with a reflux condenser. The suspension was degassed with N_2 for 15 min and then stirred under N_2 at 130°C in an oil bath for 24 h. The dark brown solution was cooled to room temperature and then poured into EtOAc (50mL). The precipitate was collected by suction filtration. The dark brown solid thus obtained was compound **W** with osmium in a 2+ oxidation state. For ease of purification, the osmium 2+ complex was oxidized to an osmium 3+ complex by dissolving the dark brown solid in 20 mL H_2O and stirring the solution in open air for 24 h. The resulting dark green solution was poured into a stirred solution of NH_4PF_6 (1g) in 20 mL H_2O . The resulting dark green precipitate of $[Os(1,1'-dimethyl-2,2'-biimidazole)_2(4-(4-carboxypiperidino)amino-2,2'-bipyridyl)]^{3+}3PF_6^-$ was collected by suction filtration and washed with 5 mL H_2O and then dried at 40°C under high vacuum for 48 h. The counter anion PF_6^- of $[Os(1,1'-dimethyl-2,2'-biimidazole)_2(4-(4-carboxypiperidino)amino-2,2'-bipyridyl)]^{3+}3PF_6^-$ was exchanged to the more water soluble chloride anion. A suspension of the PF_6^- salt of compound **W** (150mg) and Cl^- resin (10 mL) in H_2O (20 mL) was stirred for 24 h, at the end of which period all of osmium complex was dissolved. The dark green solution was separated by suction filtration and then lyophilized to give compound **W**.

The present invention should not be considered limited to the particular examples described above, but rather should be understood to cover all aspects of the invention as fairly set out in the attached claims. Various modifications, equivalent processes, as well as numerous structures to which the present invention may be applicable will be readily apparent to those of skill in the art to which the present invention is directed upon review of the instant specification.

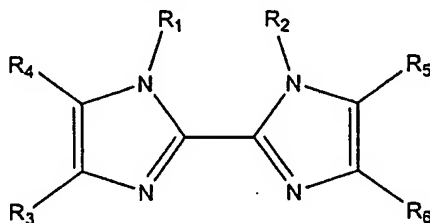
WHAT IS CLAIMED IS:

1. A transition metal complex having the formula:

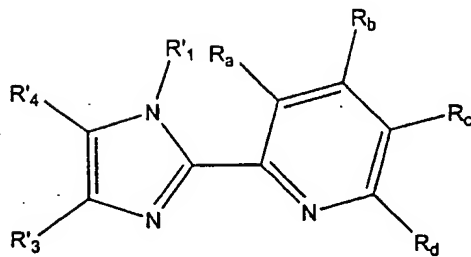


wherein M is cobalt, iron, ruthenium, osmium, or vanadium;

L is selected from the group consisting of:



and



R_1 , R_2 , and R'_1 are independently substituted or unsubstituted alkyl, alkenyl, or aryl groups;

R_3 , R_4 , R_5 , R_6 , R'_3 , R'_4 , R'_5 , R'_6 , and R'_d are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂, or substituted or unsubstituted alkoxy, carbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkoxy,

alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl;

c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge;

X represents at least one counter ion;

d is an integer from 1 to 5 representing the number of counter ions, X; and

L₁, L₂, L₃ and L₄ are ligands.

2. The transition metal complex of claim 1, wherein both R₁, R₁' and R₂ are unsubstituted C1 to C12 alkyl.

3. The transition metal complex of claim 1, wherein R₃, R₄, R₅ and R₆ are -H.

4. The transition metal complex of claim 1, wherein L₁ is a monodentate ligand.

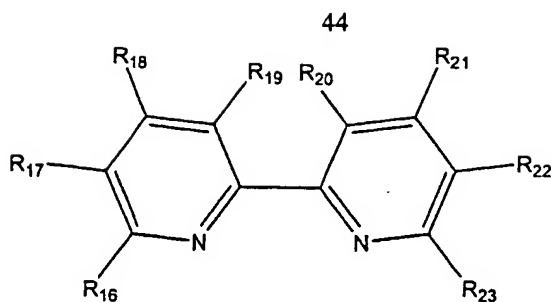
5. The transition metal complex of claim 1, wherein L₁ comprises a heterocyclic compound containing at least one nitrogen atom.

6. The transition metal complex of claim 1, wherein L₁ comprises a heterocyclic compound coupled to a polymeric backbone.

7. The transition metal complex of claim 6, wherein L₁ and L₂ in combination form a bidentate ligand.

8. The transition metal complex of claim 1, wherein L₁ and L₂ in combination form a bidentate ligand.

9. The transition metal complex of claim 8, wherein the bidentate ligand comprises a 2,2'-bipyridine having the following formula:

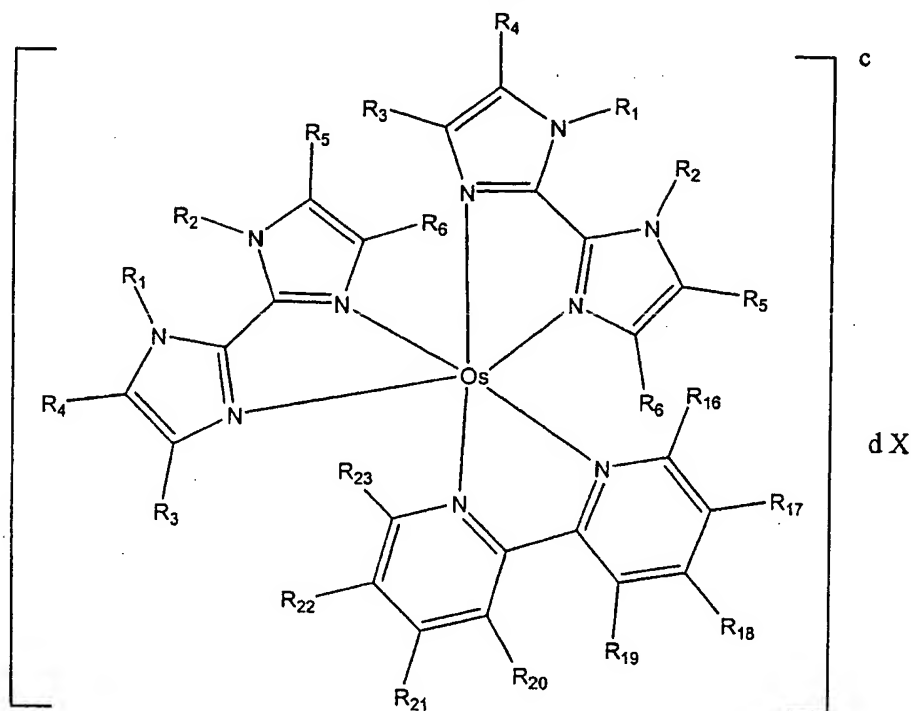


wherein R₁₆, R₁₇, R₁₈, R₁₉, R₂₀, R₂₁, R₂₂ and R₂₃ are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂, or substituted or unsubstituted alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkoxy, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxyamino, alkylthio, alkenyl, aryl, or alkyl.

10. The transition metal complex of claim 1, wherein L₁ and L₂ in combination form a first bidentate ligand and L₃ and L₄ in combination form a second bidentate ligand.

11. The transition metal complex of claim 10, wherein at least one of the first and second bidentate ligands is selected from the group consisting of substituted and unsubstituted 2,2'-bipyridines, 2,2'-biimidazoles, and 2-(2-pyridyl)imidazoles.

12. The transition metal complex of claim 1, wherein M is osmium and the transition complex has the following formula:



wherein $R_3, R_4, R_5, R_6, R_{16}, R_{17}, R_{19}, R_{20}, R_{22}$ and R_{23} are -H;

R_1 and R_2 are independently substituted or unsubstituted C1 to C12 alkyls;

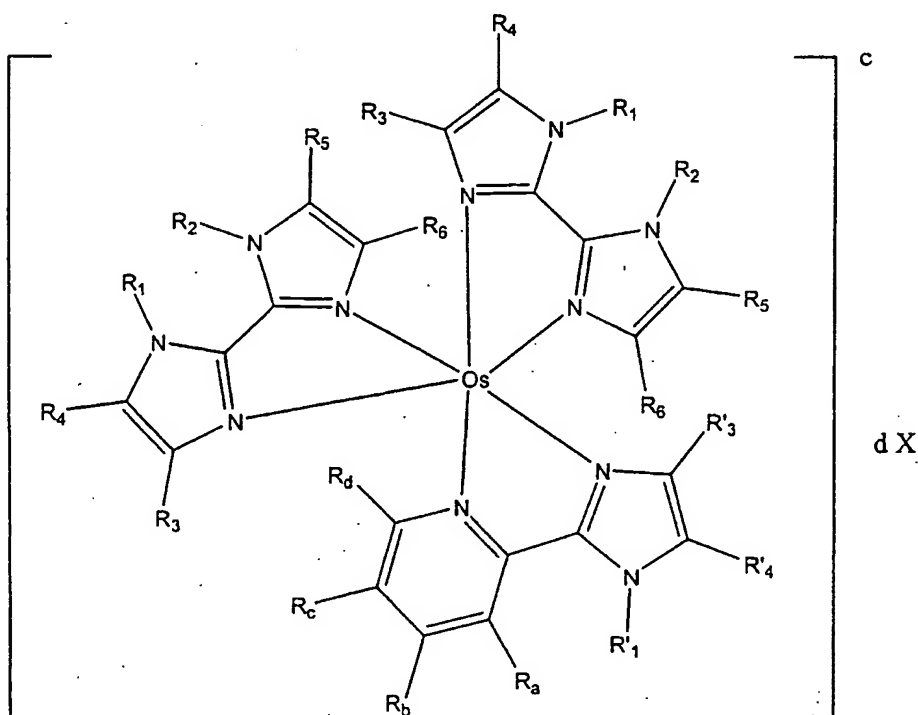
and

R_{18} and R_{21} are independently -H or substituted or unsubstituted C1-C12 alkoxy, C1-12 alkylthio, C1-C12 alkylamino, C2-C24 dialkylamino, or C1-C12 alkyl.

13. The transition metal complex of claim 12, wherein at least one of R_1, R_2, R_{18} and R_{21} comprises a reactive group selected from the group consisting of carboxy, activated ester, sulfonyl halide, sulfonate ester, isocyanate, isothiocyanate, epoxide, aziridine, halide, aldehyde, ketone, amine, acrylamide, thiol, acyl azide, acyl halide, hydrazine, hydroxylamine, alkyl halide, imidazole, pyridine, phenol, alkyl sulfonate, halotriazine, imido ester, maleimide, hydrazide, hydroxy, and photo-reactive azido aryl groups.

14. The transition metal complex of claim 12, wherein at least one of R_1 , R_2 , R_{18} , and R_{21} is coupled to a polymeric backbone.

15. The transition metal complex of claim 1, wherein M is osmium and the transition complex has the following formula:



wherein R_3 , R_4 , R_5 , R_6 , R'_3 , R'_4 , R_7 , and R_8 are -H;

R_9 is -H or methyl;

R_{10} is -H or substituted or unsubstituted C1-C12 alkoxy, C1-12 alkylthio, C1-C12 alkylamino, C2-C24 dialkylamino, or C1-C12 alkyl; and

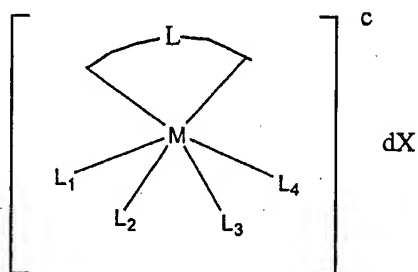
R_1 , R'_1 and R_2 are independently substituted or unsubstituted C1 to C12 alkyl.

16. The transition metal complex of claim 15, wherein at least one of R_1 , R_2 , and R'_1 comprises a reactive group selected from the group consisting of carboxy, activated ester, sulfonyl halide, sulfonate ester, isocyanate, isothiocyanate, epoxide, aziridine, halide, aldehyde, ketone, amine, acrylamide, thiol, acyl azide,

acyl halide, hydrazine, hydroxyamine, alkyl halide, imidazole, pyridine, phenol, alkyl sulfonate, halotriazine, imido ester, maleimide, hydrazide, hydroxy, and photo-reactive azido aryl groups.

17. The transition metal complex of claim 15, wherein at least one of R_1 , R_2 , and R'_1 is coupled to a polymeric backbone.

18. A redox mediator having the formula:



wherein M is iron, cobalt, ruthenium, osmium, or vanadium;

L is a bidentate ligand comprising at least one imidazole ring;

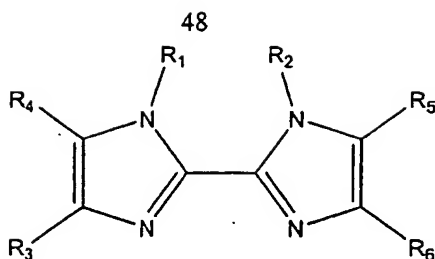
c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge;

X represents at least one counter ion;

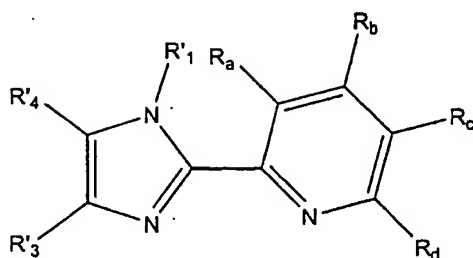
d is an integer from 1 to 5 representing the number of counter ions, X; and

L_1 , L_2 , L_3 and L_4 are ligands.

19. The redox mediator of claim 18, wherein L is selected from the group consisting of:



and



wherein R_1 , R_2 , and R'_1 are independently -H or substituted or unsubstituted alkyl, alkenyl, or aryl; and

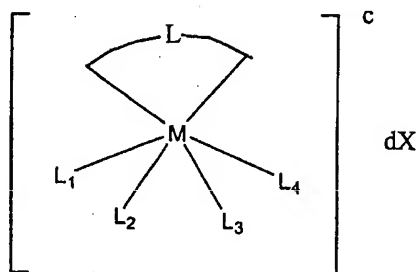
R_3 , R_4 , R_5 , R_6 , R'_3 , R'_4 , R'_5 , R'_6 , R'_a , R'_b , R'_c , and R'_d are independently -H, -F, -Cl, -Br, -I, -NO₂, -CN, -CO₂H, -SO₃H, -NHNH₂, -SH, -OH, -NH₂, or substituted or unsubstituted alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkoxy, alkylamino, dialkylamino, alkanoylamino, arylcarboxamido, hydrazino, alkylhydrazino, hydroxylamino, alkoxylamino, alkylthio, alkenyl, aryl, or alkyl.

20. The redox mediator of claim 18, wherein L_1 comprises a heterocyclic compound containing at least one nitrogen atom.

21. The redox mediator of claim 18, wherein L_1 comprises a heterocyclic compound coupled to a polymeric backbone.

22. The redox mediator of claim 18, wherein at least one of L , L_1 , L_2 , L_3 and L_4 is coupled to a polymeric backbone.

- 23 A polymer comprising:
 a polymeric backbone; and
 a transition metal complex having the following formula:

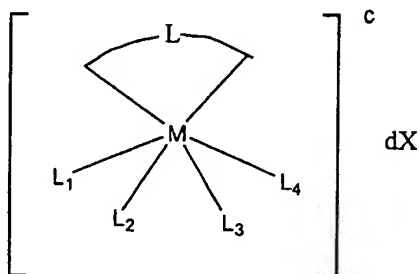


- wherein M is iron, cobalt, ruthenium, osmium, or vanadium;
 L is a bidentate ligand comprising at least one imidazole ring;
 c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge;
 X represents at least one counter ion;
 d is an integer from 1 to 5 representing the number of counter ions, X; and
 L₁, L₂, L₃ and L₄ are ligands.
 wherein at least one of L, L₁, L₂, L₃ and L₄ is coupled to the polymeric backbone.

24. The polymer of claim 23, wherein the polymer backbone is selected from the group consisting of styrene/maleic anhydride copolymer, methylvinylether/maleic anhydride copolymer, poly(4-vinylbenzylchloride) copolymer, poly(allylamine) copolymer, poly(4-vinylpyridine) copolymer, poly(4-vinylpyridine), poly(N-vinylimidazole), and poly(4-styrene sulfonate).

25. A sensor comprising:
 a working electrode;
 a counter electrode; and

a redox mediator disposed proximate to the working electrode, the redox mediator having the formula:



wherein M is iron, cobalt, ruthenium, osmium, or vanadium;

L is a bidentate ligand comprising at least one imidazole ring;

c is an integer selected from -1 to -5 or +1 to +5 indicating a positive or negative charge;

X represents at least one counter ion;

d is an integer from 1 to 5 representing the number of counter ions, X; and

L₁, L₂, L₃, and L₄ are ligands.

26. The sensor of claim 25, further comprising an enzyme disposed proximate to the working electrode.

27. The sensor of claim 25, wherein at least one of L, L₁, L₂, L₃, and L₄ is coupled to a polymeric backbone.

28. The sensor of claim 25, wherein the redox mediator is non-leachably disposed on the working electrode.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/31268

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C07F15/00 C12Q1/00 G01N27/49		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 C07F C12Q G01N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, CHEM ABS Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 35225 A (TOMASCO MICHAEL F ;HELLER ADAM (US); HELLER E & CO (US); SAY JAMES) 13 August 1998 (1998-08-13) cited in the application page 9, line 20 -page 13, line 16 page 43; example 5 page 46; claim 1 page 48; claims 15-20 page 50 -page 51; claims 41,43-50	1,18,23, 25
A	OHARA, T. J. ET AL: "Glucose electrodes based on crosslinked 'Os(bpy)2C1!+/2+ complexed poly(1-vinylimidazole) films" POLYM. MATER. SCI. ENG., vol. 70, 1993, pages 182-183, XP000981691 page 183	1,18,23, 25
<input type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>*G* document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search <div style="text-align: center; font-weight: bold;">7 February 2001</div>		Date of mailing of the international search report <div style="text-align: center; font-weight: bold;">21/02/2001</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer <div style="text-align: center; font-weight: bold;">Bader, K</div>

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/31268

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9835225 A	13-08-1998	AU 6157898 A	26-08-1998
		EP 0958495 A	24-11-1999
		JP 2000509507 T	25-07-2000
		US 6143164 A	07-11-2000
		US 6120676 A	19-09-2000

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